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A COMPARISON OF THE PHAGOCYTIC ACTION OF LEUKOCYTES IN AMPHIBIA AND MAMMALIA.[1]

JOHN M. BERRY, PETERBORO, N. Y.

The property possessed by leukocytes of ingesting foreign particles, and known as phagocytosis, is one that has excited a great deal of attention in scientific research. This property of the leukocyte is particularly interesting from a pathological standpoint. The fate of foreign matter which enters the body, the taking up of broken down blood corpuscles, the carrying of dust particles from the lungs, leading to what Ziegler has called dust diseases, and the elimination of bacteria from the body, are all phenomena depending upon this property of the corpuscle. Metschnikoff would affirm that through the phagocytic activity of the corpuscle toward infecting bacteria the body is able to resist their attack, and that it is to this property that we owe our immunity to certain diseases.

Many experiments had been performed and a great many articles had been written as to what was the fate of foreign particles introduced into the body. The methods of experiment differed in a great many cases and the results obtained were as varied and original as the methods. In 1893 Miss Claypole, then at Cornell University, devoted a part of her thesis, for the master's degree, to a discussion of this question.

Hitherto the manner of experimentation had been such as to be almost certain to bring on pathological conditions, but in Miss Claypole's work the animals were kept under normal condition as much as possible, and were subjected to such experiments only as they could meet in a physiological manner. The great fault in previous experiments had been in the quality and quantity of foreign matter used. In some cases such a heavy substance as powdered sandstone was introduced into the body, and in almost every case too much of the foreign matter was introduced. Miss Claypole used for her experiment *Necturus* and *Cryptobranchus*. The foreign matter that she used was a mixture of

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[1] This paper gives the main results of the work done in the preparation of a baccalaureate thesis in the Department of Histology and Embryology in Cornell University during the college year 1896–97. My thanks are due the department for the abundant material and facilities put at my disposal, and for the advice and encouragement from the instructing staff.

Lampblack	1 g.
Gum Arabic	1 g.
Salt	6–10 g.
Water	20 c.c.

At no time did she inject more than 1 c.c. She introduced the carbon into the jugular vein, or into the abdominal cavity.

The results of Miss Claypole's work were as follows: When the injection was made into the abdomen the carbon appeared in the circulation in from six to nine days. When the injection was made into the jugular vein the carbon appeared in the abdominal cavity. In case of abdominal injection no free carbon was found after the fourth day, and in case of venous injection after the second day; in both cases the carbon was ingested by leukocytes. In the tissues Miss Claypole found that there was:

1. No free carbon.
2. All the carbon was in leukocytes, except in the spleen.
3. Carbon-laden leukocytes extra and intravascular, except in liver, where carbon was only intravascular.
4. Ingested free cells on mucous surfaces.
5. Ingested cells with excretory products in kidney.
6. Ingested cells free in lungs.

Miss Claypole's view was that ultimately all the carbon would be removed from the body, with the possible exception of that in the spleen by the wandering out of the carbon-laden cells.

I have followed out Miss Claypole's method in my experiments, using the *Necturus* first as a comparison to her work and then going on to mammals. By reason of the facilities now possessed by the department of histology and put at my disposal, I was able to keep the animals under almost normal conditions and for a long time. I could, therefore, carry on my experiments a much longer time than Miss Claypole. She did not keep any animal more than eighteen days after injection, and while I found the same results as she did with animals kept for only that length of time, when I used animals that had been kept for a longer time I found the phenomena to be not so definite and her simple conclusions not in all cases justified. The *Necturi* used for experiment gave the following results:

NECTURUS I.

October 14, 1896, I injected about one third c.c. of the carbon mixture into the abdominal cavity. The animal was examined several times under the microscope, but no carbon-laden leukocyte could be seen circulating in the gills up to October 26th, twelve days after injection, when the animal was killed. Sections were obtained of the liver and kidney. There was no free carbon anywhere, all found being enclosed in leukocytes. The liver showed carbon at the periphery, but none toward the central part. Plate II., Fig. 2. The same was true in the kidney. In none of the sections were carbon-laden leukocytes seen in the blood-vessels.

NECTURUS II.

About ½ c.c. of the carbon mixture was injected into the abdominal cavity. Carbon-laden leukocytes were seen circulating in the gills after the seventh day. The animal was examined 100 days after injection. There was no free carbon anywhere, a very few carbon-laden leukocytes were found in the blood, while the abdominal fluid was black with carbon-laden cells. Carbon-laden leukocytes were found wandering into the tissues.

NECTURUS III. and IV. showed nothing different.

NECTURUS V.

About ½ c.c. of the carbon mixture was injected into the jugular vein. The animal was examined the day after the injection was made. The wandering leukocytes were present in the tissues. Ingested leukocytes were present in the abdominal fluid. There was no free carbon anywhere.

NECTURUS VI., which was left fourteen days, showed the same thing.

NECTURUS VII. was injected in the abdominal cavity and left twelve days. No free carbon was found and the wandering into the tissues of carbon-laden leukocytes could be seen. In the liver the ingested cells were present in the blood and between the liver cells proper. The carbon was very abundant along the periphery of one edge, while that of the opposite edge was almost free. This is explained by the fact that the edge which showed the abundance of carbon is on the dorsal side of the liver, where the abdominal fluid freely bathes it.

NECTURUS VIII. showed nothing different.

NECTURUS IX.

About ½ c.c. was injected into the jugular vein. Examination made six days later. Carbon-laden leukocytes were found in the abdominal fluid and in the blood. No free carbon was found. The dorsal aorta was exposed and tied cephalad of the point where the splenic arteries are given off. A 6-10 per cent. chloral solution was then forced cephalad into the aorta. By this means the blood-vessels of the spleen and liver were washed out, but that this might be more thorough the liquid was also forced in the opposite direction through the portal vein. Picric alcohol was then forced through to insure thorough fixation of the parts. Sections were made of the liver and spleen. In the liver the appearance was that there were carbon-laden leukocytes outside the capillaries and also carbon in pigment cells. (Plate I., Fig. 2.) In the spleen the carbon seemed to have the same relation to the tissues as Miss Claypole described.

NECTURUS X. was injected in the abdominal cavity and left for 164 days. Carbon-laden leukocytes were found in the abdominal fluid, but not in the blood. No further examination was made.

The results in regard to experiments on *Necturus*, as can be seen from the above, are similar to those of Miss Claypole when the animals are kept alive for only a short time. When carbon was injected into the abdominal cavity it made its appearance after some days in the blood enclosed in leukocytes, and when, on the other hand, the injection was made into the jugular vein the carbon-laded leukocytes appeared in the abdominal fluid. The apparent wandering out of the carbon-laden cells in the lungs, intestinal tract and kidney was found even in *Necturus* V., which had been injected in the jugular vein for only one day when examined. Transfer of the carbon from the blood to the lymph had also taken place in this short time since carbon-laden cells were found in the abdominal fluid. It is interesting to note that in *Necturus* I., although carbon had not appeared in the blood, it was found in the periphery of the liver. More will be said in regard to this later. The liver and spleen are organs in which the relation of the carbon to the tissues is very difficult to make out. Miss Claypole found in the liver that the carbon was present only in leukocytes and that these were always intravascular. Many of my sections would seem to show that not only was carbon present in leukocytes which were extravascular, but in one case (*Necturus* IX.) the appearance was that carbon was present in the pigment cells. The fact that in *Necturus* II., while but a very small amount of carbon could be found in the blood, yet a section of the liver showed a comparatively large amount of carbon would seem to show that some of the carbon present in the liver was extravascular.

It is only when animals are examined that have been left for a considerable time after injection that conditions are met with that do not correspond with Miss Claypole's conclusion, that the carbon-laden leukocytes would all wander from the body. In *Necturus* II., from the condition found in animals examined only a short time after injection, one would expect that all of the carbon, with the possible exception of that in the spleen and liver, would be out of the body. The actual fact was, however, that the greater part of the carbon was still present. Why there should be such a large amount of carbon still in the abdominal cavity, while there was such a small amount in the blood, is a fact for which I can find no explanation. A great quantity of carbon was also collected under the peritoneum in the connective tissue of the abdominal wall.

The same methods were employed for the experiments on mammals as on *Necturus*. The exact relation in the liver and spleen was not attempted to be worked out. The results are as follows:

Rat I., II. and III. injected with about 1.2 c.c. of carbon mixture into the abdominal cavity and left two, seven and fifty-nine days respectively. No free carbon was found in either case. Essentially the same phenomena were found as in *Necturus*. In Rat II., while carbon was found in blood, tissues and abdominal cavity, there was none in an abdominal lymph gland. In Rat III., while much carbon was present in the abdominal fluid, there was none in blood and but a small amount in the tissues. In Rat III. the carbon had collected in large quantities beneath the peritoneum.

Rat IV. was injected in the jugular vein. Carbon-laden leukocytes were found in the abdominal and thoracic fluids the next morning. The carbon-laden cells had also wandered into the tissues somewhat.

RAT V.

Animal was fed a mixture of cream and egg into which carbon mixture had been stirred. The animal was examined the next day. Portions of the stomach, small intestine, cecum and large intestine were taken. Some preparations were fixed in osmic acid to show the fat absorption, while others were fixed in ordinary fixers and stained to show the carbon. It was seen that fat absorption took place, but in no section could I find any carbon within the tissue, but in every case, and especially in the stomach, the appearance was that some of the carbon was enclosed in leukocytes. Plate V., Fig. 2.

RAT VI.

Animal was placed in a glass jar and the air in the jar filled with a fine suspension of lampblack by means of an insect powder spray. The animal was allowed to breathe this for about three-quarters of an hour. The animal was examined the next day. Sections were made only of the lungs. Carbon was found in leukocytes, free in the alveoli and also in the walls of the infundibula. Plate V., Fig. 1.

An interesting result of experiment is shown in Cat I. In this case the injection was intended to be made into the abdominal cavity, but the needle did not penetrate far enough and the carbon was injected into the subcutaneous tissue. The animal was examined after three days. A large quantity of the carbon was found to be free along the inner surface of the skin, but a large amount had been taken up by leukocytes and carried away, following, in great part, connective tissue bundles. Plate II., Fig. 3.

DOG I.

Animal injected with about 1 c.c. into the abdominal cavity. Examined after two days. Very little free carbon. Carbon laden cells were present in the abdominal and thoracic cavities. No carbon was found in the blood, although carbon was found in the lymph glands of the abdomen.

DOG II.

Animal injected with about 1.2 c.c. in the abdominal cavity. Examined after fifteen days. No carbon-laden cells were found in the blood or thoracic cavity, but these were present in the abdominal cavity. There was no free carbon. Carbon-laden cells were wandering into the tissues and were found free on mucous surfaces. The abdominal lymph glands contained comparatively little carbon. The carbon was very abundant in the connective tissue of the abdominal wall.

RABBIT I.

Injection made into a vein of the ear. Animal was left fifteen days. No free carbon was found. No carbon-laden cells were found in blood, but these were present in abdominal and thoracic fluids.

From the results of the above experiments it will be seen that essentially the same phenomena occur in the mammals as in the *Amphibia.* When the injection was made into the abdominal cavity of a mammal the foreign particles were quickly ingested by the leukocytes and in a short time these leukocytes made their appearance in the blood and in the tissues of the body. When the injection was made into a vein the carbon-laden cells appeared in the abdominal cavity. This shows that as in *Amphibia* transfer takes place from the lymph to the blood and from the blood to the lymph. The wandering out upon mucous surfaces of carbon-laden cells is found to be common to both *Amphibia* and *Mammalia.* In the *Necturus* it was found that the carbon was, to all appearances, outside of the blood-vessels in the liver. This seems to be true also with the mammals, for when no carbon could be found in the blood, sections of the liver showed the presence of much carbon. Carbon was always found in the spleen, but no attempt was made to work out its exact relation to the organ ; it was noted, however, that there was never any carbon within the malpighian corpuscles.

In mammals as in *Amphibia*, when the animal is left alive for some time after the injection it is found that a large amount of carbon is still in the body. In mammals the collection of carbon under serous membranes and in connective tissue is especially marked. (Plate II., Fig. 1.)

Experiments were performed on mammals that showed that the action of the leukocytes toward foreign matter is even more complicated than was found in previous experiments on *Necturus*. The leukocytes act both ways, for not only do they remove foreign matter from the body to a certain extent, but they also bring it into the body from the lungs and possibly the intestinal tract.

Only one experiment was performed to determine whether foreign matter could enter the body through the alimentary canal. The result of the experiment was that while no carbon was found in the tissues of the intestine, yet cells containing carbon were found in the lumen of the canal. Whether or not these leukocytes would again enter the tissue of the enteron can only be determined by further experimentation.

On Rat VI. an experiment was performed to throw some light on a point over which there have been numerous discussions, viz: Whether foreign matter enters the body through the lungs, and if so, how this takes place. Dr. Arnold, who has done the most work in this line, came to the conclusion that the lymph glands act as filters. Others believe that the carbon enters the lymph stream free through preformed paths. The trouble with all investigations was in the manner of experiment. Arnold in many cases caused the inhalation of so much foreign matter that the animal died, and other experimenters even introduced the foreign matter into the lungs suspended in a liquid; it is not to be wondered at that pathological phenomena would result. While I have performed only one experiment along this line and consequently cannot hope to solve the problem, yet carrying out the experiment as nearly under normal conditions as I could and getting into the lungs comparatively only a small amount of carbon, I feel that what took place was physiologically true. The results show that carbon enters the tissue of the lung. No carbon enters the tissue that is not enclosed in cells. I could determine whether, as Arnold said, the lymph glands acted as filters only by further experiment; but they certainly do not in the elimination of carbon-laden leukocytes from the abdominal cavity and I do not see why they should here.

The experiment on Rabbit I. shows that transfer of carbon from the blood to the lymph takes place. This is comparable with the results found in *Necturus*.

When now a comparison is made between *Amphibia* and *Mammalia* it is seen that in the main the phenomena are the same. In neither case was free carbon found anywhere in the tissues. The fact that so many experimenters report free carbon may be due to the collection of carbon at the connective tissue nodes. And it is very possible that the appearance of free carbon is given by the tearing of the knife in making the sections.

In *Necturus* I., before carbon-laden leukocytes had made their appearance in the blood, sections of the liver and kidney showed carbon at the periphery. This shows that carbon-laden leukocytes can leave the abdominal cavity without first entering the blood. In mammals it was found that when the tissues around the abdominal cavity as well as the blood contained great quantities of carbon the lymph glands were comparatively free. This shows that the carbon laden leukocytes can leave the abdominal cavity without first entering the lymph. The results in both *Amphibia* and mammals show, therefore, that the carbon does not wholly enter the blood and, on the other hand, does not wholly enter the lymph stream; but when leaving the abdominal cavity enclosed in leukocytes, the leukocytes wander where they will. It would further seem that those escaping from the body do so only by chance, for if the object of the leukocyte was to rid the body of foreign matter why should it carry foreign matter from the lungs into the body? In both *Amphibia* and *Mammalia* more carbon is found in the spleen following intravenous injection than after abdominal injection. This would seem to show that the carbon that is in the spleen comes through the blood and not the lymph. In the liver, however, carbon appears in the periphery before it is found in the blood (*Necturus* I.). This difference may be due to the greater density of the capsule of the spleen. The finding of carbon-laden leukocytes in the abdominal fluid in the case of *Necturus* II., three months, and in the case of Rat III., two months after injection, is not easy to explain. It is hard to see why they should be there in any case; but since they were there their presence might indicate that the leukocytes lived three months at least. It may be, however, that before wandering out, the leukocyte has degenerated and the carbon has been taken up by fresh leukocytes.

As to what will be the ultimate fate of the carbon can be told only by continued experimentation and extended time. As far as my experiments have carried the carbon has been always found in leukocytes with the exception of the splenic pulp cells and possibly the pigment cells of the liver. How the carbon finds its way into these is a question yet to be solved. The changing of a leukocyte to a fixed cell is a phenomenon that has never been seen and for which there is no proof. I found nothing that would indicate this in any of my sections. I simply noted the tendency for the carbon to collect under serous membrane, but can offer no explanation for it.

GENERAL SUMMARY.

1. The phenomena in *Amphibia* and *Mammalia* are essentially the same.
2. No free carbon in tissues.
3. Carbon passes from lymph to blood.
4. Carbon is transferred from blood to lymph.

5. There is no special road by which the carbon leaves the abdomen.

6. Foreign matter escapes from the body through lungs, intestinal tract, kidney and skin.

7. Leukocytes that wander out do so by chance.

8. The situation of the carbon was the same as that found by Miss Claypole with the exception of the liver where the carbon is outside of the blood-vessels in my experiments.

9. Foreign matter may enter the body through the lungs and possibly through the intestines.

10. The carbon-laden cells tend to collect under serous membrane.

METHODS.

In addition to the general methods already spoken of, it may be well to give some of the details that were used in preparing the specimens for examination. For fixing and hardening the tissues the picric-alcohol method, as used by Prof. Gage, was found to be the simplest and most effective when only the general structure of a part was desired. The whole animal could be put into this fixing agent, but the best results were obtained when small pieces of the tissue to be examined were cut out and fixed. It was found advisable in either case to change the liquid at least once. When the relations of cells and minute structures were to be worked out a saturated normal salt solution of mercuric chloride, to which one per cent. of acetic acid was added, was found to be the best fixer. Care must be taken in the use of this to wash out all of the mercuric chloride or there will be black crystals in the tissues.

Tissues were sectioned by both the collodion and paraffin methods. The collodion method employed was that used by Prof. Gage. Most of the sections were made by the paraffin method and it was easier to make serial sections.

A large amount of pigment was found in the tissues of *Necturus*. To remove this the sections, when cut and fastened to the slide, were placed in a jar of hydrogen dioxide (10 Vol. or 2 per cent. solution), the pigment became light yellow in a few hours. The process was hastened by placing in sunlight. This could not be done when the sections were cut in collodion and fastened to the slide by ether-alcohol since the hydrogen dioxide seemed to act on the collodion and loosen it from the slide. Very little trouble was found when the sections were cut in paraffin and fastened to the slide with albumen fixative.

A stain of hematoxylin and Van Gieson's picro-fuchsin was found to give the best results. The sections were first stained with hematoxylin and then with the picro-fuchsin, the formula for which is :

1 per cent. aqueous solution acid-fuchsin, 5 c.c.
Saturated aqueous solution picric acid, 100 c.c.

The specimen should be mounted in acid balsam after using this stain, *i. e.*, in balsam which has not been neutralised.

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No attempt has been made to give a complete bibliography of the articles written that apply to this subject. A bibliography of 264 works can be found in Arnold's "Untersuchungen über Staubinhalation und Staubmetastase." Several articles not mentioned by Arnold can be found in Miss Claypole's bibliography.

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PLATE I.

Fig. 1. Transection of small intestine of *Necturus* VI., drawn with the camera lucida from a single field of the microscope; the carbon-laden cells can be seen within the blood-vessel and wandering out through the tissues toward the lumen.

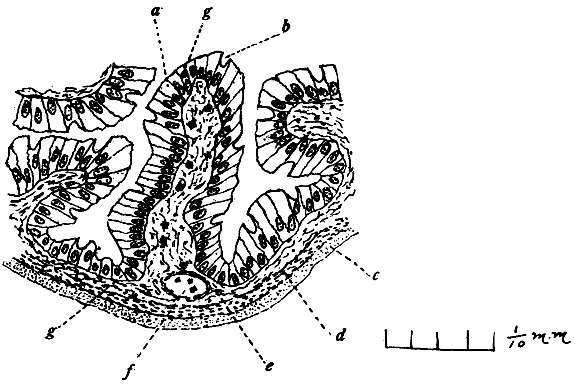
(*a*) epithelial cells.
(*b*) goblet cells.
(*c*) longitudinal muscular coat.
(*d*) circular muscular coat.
(*e*) connective tissues.
(*f*) blood-vessel.
(*g*) carbon-laden leukocytes.

Fig. 2. Section of the liver of *Necturus* IX.

(*a*) liver cell.
(*b*) pigment cell of liver containing granules of carbon.
(*c*) leukocytes containing carbon.

PLATE I.

1



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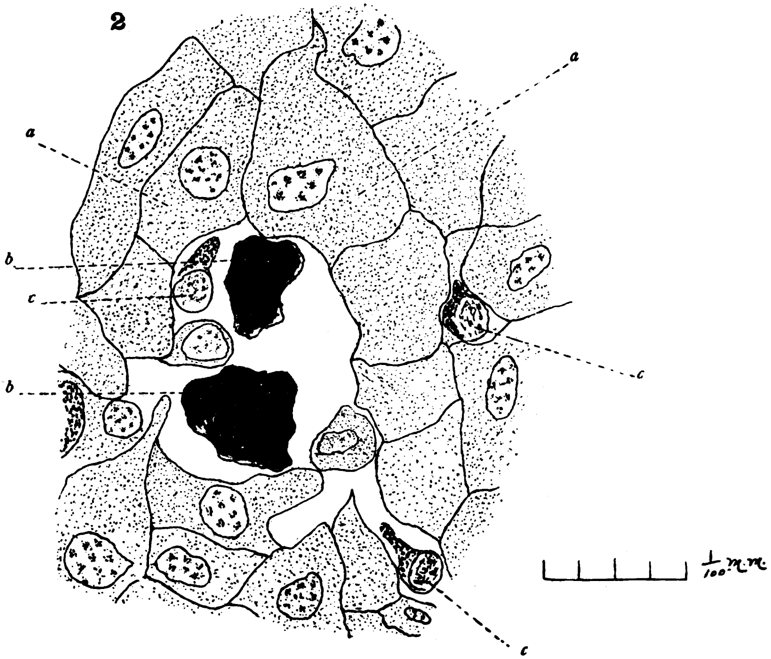

PLATE II.

Fig. 1. Section of abdominal wall of Rat III.
- (*a*) muscle bundles.
- (*b*) peritoneum.
- (*c*) groups of carbon-laden leukocytes.

Fig. 2. Section of liver of *Necturus* I.
- (*a*) liver cell.
- (*b*) pigment cell.
- (*c*) capillary.
- (*d*) bile capillary.
- (*e*) carbon-laden leukocytes.

Fig. 3. Section through the skin of Cat I.
- (*a*) epidermis.
- (*b*) section of hair follicle.
- (*c*) corium.
- (*d*) adipose tissue.
- (*e*) blood-vessel.
- (*f*) connective tissue.
- (*g*) carbon-laden leukocytes. Note the free carbon in the connective tissues beneath the adipose tissue.

PLATE II.

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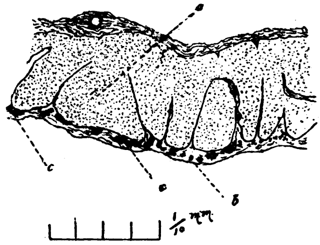

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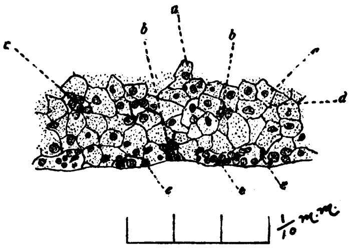

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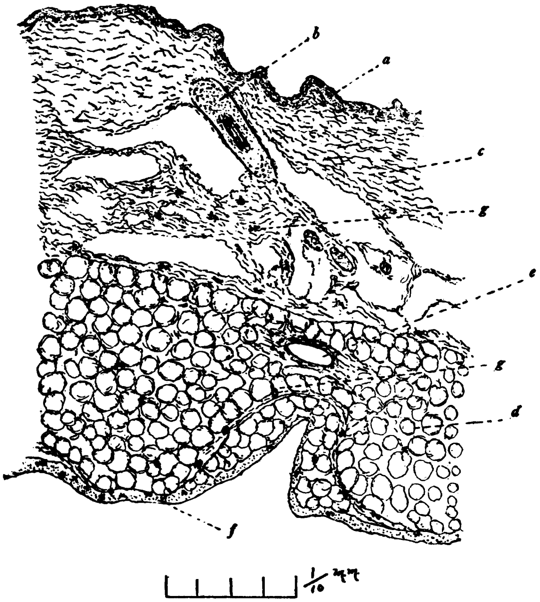


PLATE III.

Fig. 1. Section of stomach of Dog II.

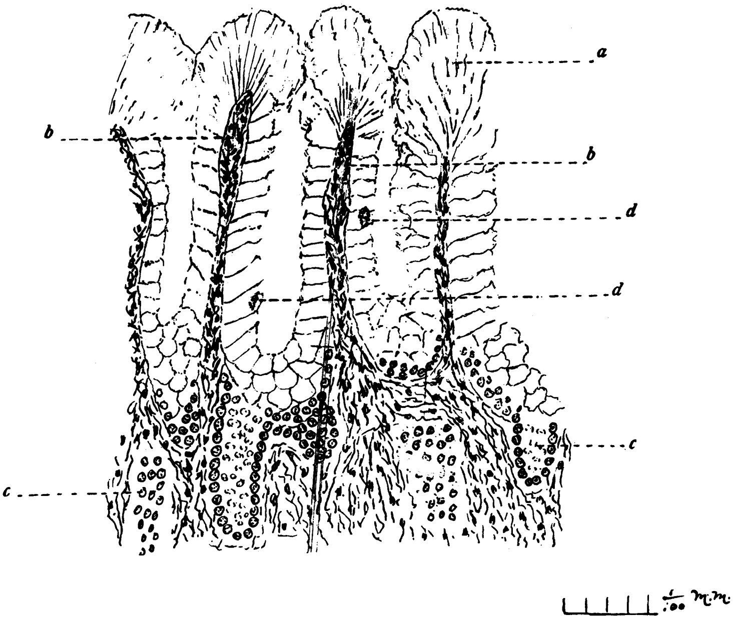
- (*a*) follicle.
- (*b*) connective tissue.
- (*c*) gland.
- (*d*) carbon-laden leukocytes wandering out.

Fig. 2. Section of lung of Dog II.

- (*a*) infundibula.
- (*b*) walls of infundibula.
- (*c*) blood-vessel.
- (*d*) carbon-laden leukocyte.

PLATE III.

1



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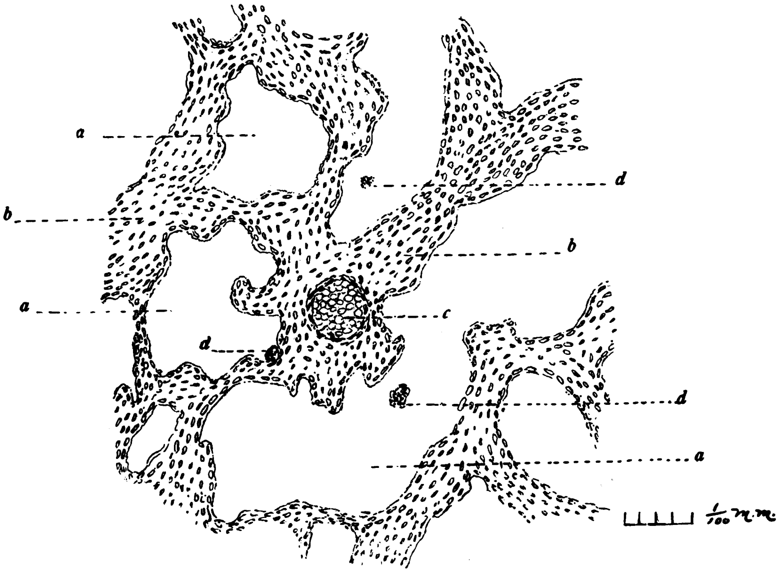


PLATE IV.

Fig. 1. Section through the diaphragm of Dog II.

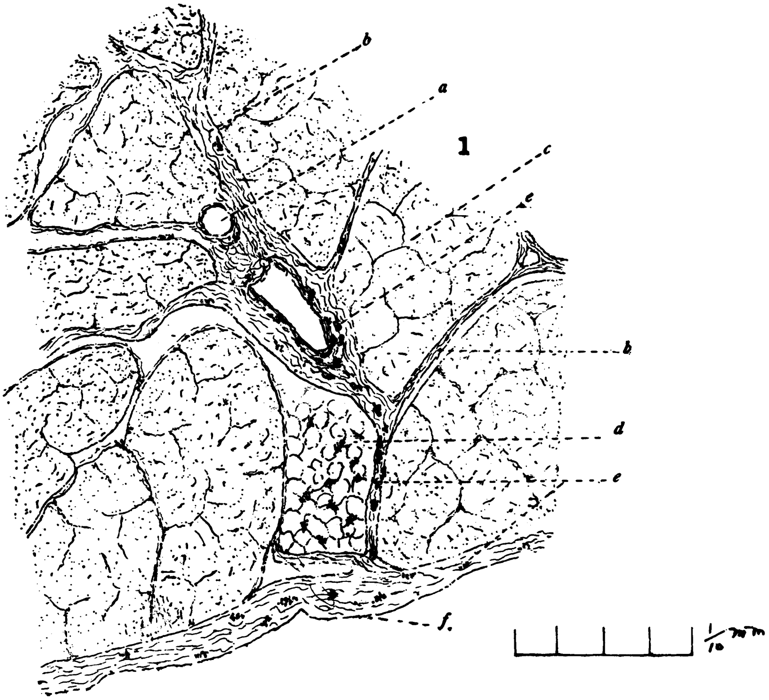
- (*a*) blood-vessel.
- (*b*) connective tissue.
- (*c*) muscle bundles.
- (*d*) adipose tissue.
- (*e*) carbon-laden leukocytes.
- (*f*) the abdominal surface.

Fig. 2. Portion of the urinary tubule from kidney of Dog. I.

- (*a*) lumen of tubule.
- (*b*) cell of wall of tubule.
- (*c*) carbon-laden leukocyte in lumen of tubule.

PLATE IV.

1



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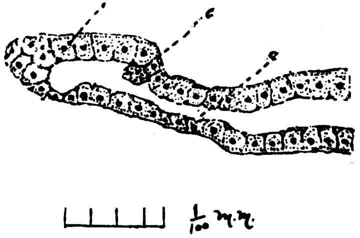

PLATE V.

Fig. 1. Section of lung of Rat VI.
  (*a*) infundibula.
  (*b*) section of bronchiole.
  (*c*) walls of infundibula.
  (*d*) carbon-laden leukocytes.

Fig 2. Section through the villi from intestine of Rat V.
  (*a*) epithelial cells
  (*b*) connective tissue.
  (*c*) goblet cell.
  (*d*) carbon-laden leukocytes.
  (*e*) uningested carbon free in lumen of intestine.

PLATE V.

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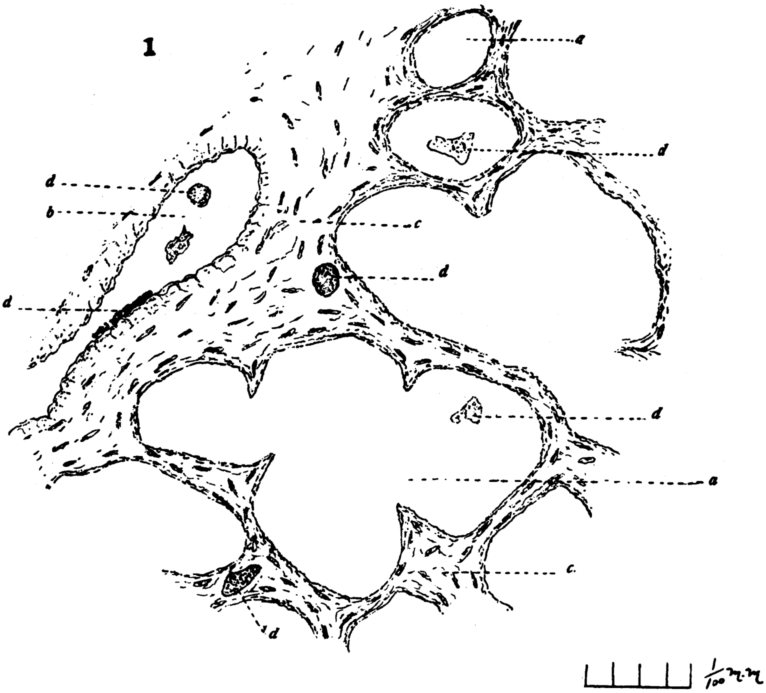

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